Genes & Genomics (2021) 43:399–406
https://doi.org/10.1007/s13258-021-01063-6

Online ISSN 2092-9293
Print ISSN 1976-9571

RESEARCH ARTICLE

Identification of QTLs associated with the anaerobic germination potential using a set of *Oryza nivara* introgression lines

Licheng Liu[1,2,3] · Xiaoxiang Li[2,3] · Sanxiong Liu[2,3] · Jun Min[2,3] · Wenqiang Liu[2,3] · Xiaowu Pan[2,3] · Baohua Fang[2,3] · Min Hu[2,3] · Zhongqi Liu[1] · Yongchao Li[2,3] · Haiqing Zhang[1]

Received: 6 December 2020 / Accepted: 9 February 2021 / Published online: 20 February 2021
© The Genetics Society of Korea 2021

Abstract

Background Rice (*Oryza sativa* L.) is an important crop and a staple food for half of the population around the world. The recent water and labor shortages are encouraging farmers to shift from traditional transplanting to direct-seeding. However, poor germination and slow elongation of the coleoptile constrains large-scale application of direct-seeding.

Objective Thisstudy was aimed to investigate the genetic basis of the anaerobic germination(AG) potential using a set of *Oryza nivara* (*O. nivara*) introgressionlines (ILs).

Methods Inthis study, a total of 131 ILs were developed by introducing *O. nivara* chromosomesegments into the elite indica rice variety 93-11 through advanced backcrossingand repeated selfing. A high-density genetic map has been previouslyconstructed with 1,070 bin-markers. The seeds of ILs were germinated and usedto measure coleoptile length under normal and anaerobic conditions. QTLsassociated with AG potential were determined in rice.

Results Basedon the high-density genetic map of the IL population, two QTLs, *qAGP1* and *qAGP3* associated with AG tolerance were characterized and locatedon chromosomes 1 and 3, respectively. Each QTL explained 15% of the phenotypic variance.Specifically, the *O. nivara*-derived chromosomesegments of the two QTLs were positively tolerance to anaerobic condition byincreasing coleoptile length. In a further analysis of public transcriptomedata, a total of 26 and 36 genes within *qAGP1* and *qAGP3* were transcriptionallyinduced by anaerobic stress, respectively.

Conclusions Utilizationof *O. nivara*-derived alleles at *qAGP1* and *qAGP3* can potentially enhance tolerance to anaerobic stress at thegermination stage in rice, thereby accelerating breeding of rice varieties tobe more adaptative for direct-seeding.

Keywords *Oryza nivara* · Introgression line · Anaerobic germination tolerance · QTL

Introduction

Rice is one of the most important crops in the world. Due to the shortage of labor, rice planting mode have been profoundly shifted from transplanting to direct-seeding in the last decade in China. However, direct-seeding has its disadvantages that plants are typically exposed to low temperatures, anaerobic stress, competing weeds and other adverse factors (Farooq et al. 2011). Among them, anaerobic stress beginning at the germination and early-seedling stages is the primary environmental stress associated with direct-seeding that limits the germination rate, seedling uniformity, and consequently grain yield in rice.

Rice is grown in flooded conditions and thus exhibits greater tolerance to submergence than other crops such as maize and wheat (Hattori et al. 2011). The tolerance of rice seedlings to anaerobic stress at the germination stage is a complex quantitative trait controlled by multiple genetic loci. In previous studies, numerous QTLs associated with tolerance to anaerobic stress at the germination and early-seedling stages have been identified using linkage and association mapping populations (Jiang et al. 2006; Angaji et al.

✉ Yongchao Li
yongchao_li128@163.com

✉ Haiqing Zhang
hunanhongli@aliyun.com

[1] College of Agriculture, Hunan Agricultural University, Changsha 410128, China

[2] Hunan Rice Research Institute, Hunan Academy of Agricultural Science, Changsha 410125, China

[3] MOA Key Laboratory of Indica Rice Genetics and Breeding in the Middle and Lower Reaches of Yangtze River Valley, Changsha 410125, China

2010; Septiningsih et al. 2013; Baltazar et al. 2014; Hsu et al. 2015; Angaji 2008; Zhang et al. 2017). For example, Jiang et al. (2006) reported two putative QTLs associated with AG potential using an F_2 segregation population derived from a cross between USSR5 (*japonica* subspecies) and N22 (*indica* subspecies). Five putative QTLs for flooding tolerance were detected using a BC_2F_2 population with IR64 (*japonica*) as a recurrent parent and Kho Hlan On (*japonica*) as a donor parent (Angaji et al. 2010). A study that examined coleoptile lengths of 432 *indica* varieties cultivated under normal (un-flooded) and flooded conditions, detected 2 and 11 significant SNPs associated with tolerance to flooded condition (Zhang et al. 2017). Several QTLs were further cloned using map-based cloning method. For example, *OsTPP7* at the *qAG-9-2* region, which is involved in trehalose-6-phosphate metabolism, confers AG tolerance (Kretzschmar et al. 2015; Ye et al. 2018) found that the sequence variations in *OsCBL10* promoter between upland (Up221, flooding-sensitive) and lowland (Low88, flooding-tolerant) varieties might contribute to their differentiation in flooding tolerance. Results from these studies provide important genetic information for the molecular breeding of rice varieties with AG potential.

Low oxygen under anaerobic condition alters plant metabolism, and consequently affects plant growth. One of the common metabolic responses to low oxygen is alterations in the glycolysis pathway, as evidenced by the up-regulated activities of enzymes (e.g. amylases, phosphofructokinase, fructose-6-phosphate-1-phosphotransferase, alcohol dehydrogenase, and pyruvate dehydrogenase) in plants grown under hypoxic conditions (Gibbs et al. 2000; Kato-Noguchi et al. 2007; Lasanthi-Kudahettige et al. 2007; Magneschi et al. 2008; Kretzschmar et al. 2015; Loreti et al. 2016; Loreti et al. 2018; Fukao et al. 2019). A large number of transcriptomic analysis were performed to explore the molecular mechanisms involved in regulating the growth of rice coleoptile under hypoxic and anoxic conditions (Shingakiwells et al. 2011; Narsai et al. 2015). These studies determined a complex mechanism associated with coleoptile growth, which includes carbohydrate metabolism, fermentation, hormone induction, cell division and expansion.

Wild rice is the progenitor of cultivated rice and comprises a primary gene pool for the improvement of cultivated rice. Due to the continual long-term natural selection, wild rice carries favorable alleles resistant to many abiotic and biotic stresses. To identify and utilize the favorable alleles from wild rice, we investigated a morphological trait, coleoptile length, responsible for AG potential in rice seedlings. We used 131 ILs derived from an advanced backcross between *O. nivara* (the donor parent), and *indica* rice 93-11 (the recipient parent) (Ma et al. 2016). Based on a high-density genetic mapping, two QTLs associated with AG potential were detected, one located on chromosome 1 and the other on chromosome 3. Notably, the *O. nivara*-derived chromosome segments at the two QTLs enhanced AG potential at the germination stage of seedlings in the background of 93-11. Altogether, our findings not only provide new genetic resources from wild rice for breeding rice varieties with more AG potential, they can also be used to help clone genes conferring AG potential in rice.

Materials and methods

Plant material

131 ILs used in this study were generated in a previous study (Ma et al. 2016). The recipient parent 93-11 (indica rice variety) is widely grown in China. The donor parent W2014 is an annual wild rice accession (*O. nivara*) originated from India (20° 18′ N, 72° 55′ E) and kept at the National Institute of Genetics, Japan. To develop the IL population, single plant was selected from the recipient parent 93-11 and the donor parent *O. nivara*, respectively, for crossing. A total of 23 F_1 plants were obtained. All F_1 plants were backcrossed three times in succession to 93-11 to generate BC_3F_1 population including 256 individuals. Based on the genotypes of BC_3F_1 generated by 120 polymorphic SSR markers across the whole genome, total 150 BC_3F_1 were selected and self-pollinated for six generations to generate an IL population with 131 lines.

Measuring phenotype

Two independent experiments (in September 2019 and November 2019) were done to measure 131 ILs. Each experiment was designed as follows. Seeds were dried at 50 °C for 72 h to break dormancy. Then seeds were sterilized with sodium hypochlorite (1.5%) for 15 min and rinsed eight times with sterile distilled water. A normal and an anaerobic treatment were established. For the normal condition, fifteen sterilized seeds for each line were germinated in five un-capped glass tubes (10 ml; three seeds per glass tubes) containing sterilized filter paper and 1 ml of water. For the anaerobic condition, fifteen sterilized seeds for each line were germinated in five capped glass tubes (10 ml; three seeds per glass tubes) with 5 ml of water (up to 5 cm in the 10-cm tube). All tubes for both treatments were placed in a growth chamber at 28 °C for 7 days in the dark. The coleoptile length was measured from 1 day to 7 day using an ordinary ruler. Statistical analysis was performed with SAS (Statistical Analysis System, version 8.01) and Microsoft Excel.

QTL mapping

SNPs were called from whole-genome resequencing data of ILs and parents by the software BWA (Li et al. 2009a) and SAMtools (Li et al. 2009b), using the default parameters. A sliding-window analysis was applied to genotype the ILs and a high-quality linkage map of 1070 bins was constructed (Ma et al. 2016). The detail method was described in a previously published paper (Ma et al. 2016).

The averaged value of coleoptile length across 15 seeds for each line was used to detect QTLs involved in AG potential. Missing values of phenotype were excluded from our analysis. QTL mapping was conducted using both single marker regression and composite interval mapping (CIM) method by IciMapping V4.0 software (Li et al. 2007). For CIM, the method used was the stepwise cofactor selection, in which markers were used as cofactors, and maximum number of cofactors was selected automatically. Significant threshold values of LOD scores were determined using permutation tests (Churchill et al. 1994) with 1000 replicates. The Type I error to detect the LOD threshold was defined at $P <= 0.05$. Finally, the statistical threshold was LOD > = 3.

QTL comparisons

The QTLs detected in this study were compared with previously published QTLs. The markers were placed on the physical map by BLAST software (http://blast.ncbi.nlm.nhi.gov/Blast.cgi) aligned against the reference rice genome. If no sequence information were detected, the flanking markers around the peak were used to serve as new guide.

Results

Phenotype evaluation of ILs under normal and anaerobic conditions at the germination stage

The fast growth rate of rice coleoptile helps seedling escape the anoxic environment, which improves the survival rate of rice. Therefore, rice coleoptile is a classical organ used for assessing AG potential. To identify the favorable alleles from wild rice conferring more AG potential, we measured the coleoptile length of plants at the germination stage of 131 ILs and their parents cultivated under normal and anaerobic conditions (Table S1).

Under anaerobic condition, 93-11 was germinated faster than *O. nivara*. The coleoptile of 93-11 was observed on the first day, while the coleoptile of *O. nivara* was seen on the second day. Although the coleoptile of 93-11 emerged earlier than that of *O. nivara*, the coleoptile length, coleoptile surface area and coleoptile volume of *O. nivara* were significantly greater than that of 93-11 at the end point (7th day). At the 3th day, coleoptile length of *O. nivara* increased faster than that of 93-11. In particular, coleoptile growth of 93-11 mainly occurred from the 2th to the 3th day, while coleoptile growth of O. *nivara* occurred between the 2th and 5th day.

The coleoptile length of ILs under normal condition (CLN) ranged from 1.04 to 2.17 cm and that of ILs under anaerobic condition (CLA) ranged from 0.10 to 2.40 cm (Fig. 1a–d). An anaerobic potential index (API) was calculated by the equation (CLA CLN) / CLN. Values of API ranged from − 0.94 to 0.60 among 131 ILs (Fig. 1e, f), where low values signify low AG potential and high values signifies high AG potential. A total of 109 ILs produced longer coleoptiles than that of the recipient parent 93-11 (0.18 cm) and 102 ILs had higher API values than the value of the recipient parent 93-11 (− 0.87) (Table S1). These data suggest that the chromosomal segments from wild rice may contain favorable alleles for enhancing AG potential. The averaged coleoptile length of ILs was shorter under the anaerobic condition than that under the normal condition (Table S1). Additionally, the Pearson correlation coefficient (PCC) of coleoptile length under the normal and anaerobic conditions was 0.04 ($p > 0.05$) (Supplemental Fig. 1), implying

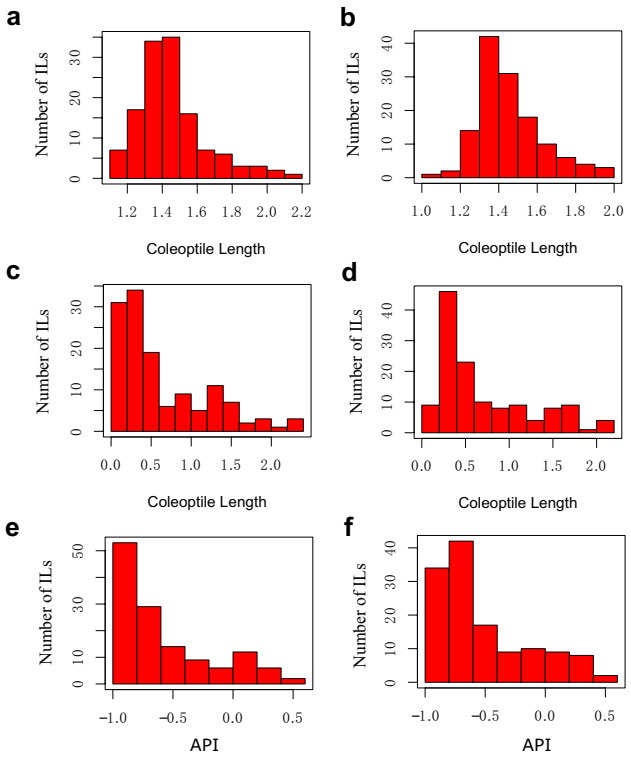

Fig. 1 Distribution of coleoptile length under normal and anaerobic condition in 131 ILs. Coleoptile length of rice for replicate 1 (**a**) and replicate 2 (**b**) under normal condition (CLN). Coleoptile length of rice for replicate 1 (**c**) and replicate 2 (**d**) under anaerobic condition (CLA). Anaerobic potential index (API) for replicate 1 (**e**) and replicate 2 (**f**). The x-axis represented coleoptile length (centimeter)

that different genetic loci were associated with coleoptile growth under normal and anaerobic conditions.

QTL mapping for AG potential using 131 ILs

Our previous study reported a high-density genetic map of a set of *O. nivara* ILs through high-throughput whole-genome resequencing. This genetic map contained 1070 bin-markers, where a bin had an average length of 349 kb. To identify QTLs associated with AG potential, QTL analysis was conducted using both the single marker regression and CIM. QTLs detected in both methods were robust and used for following analysis. Two independent experiments were done in September 2019 and November 2019. For each experiment, coleoptile length under normal and anaerobic conditions were measured, respectively. Under the normal condition, QTL detected in the two experiments was co-located (Fig. 2a). *qCLN1* (*Coleoptile Length under the Normal Condition 1*), was located in marker bin150 on the long arm of chromosome 1 and explained 43 % and 44 % of phenotypic variance for replicate 1 and replicate 2, respectively (Table 1; Fig. 2a). The location of bin150 was located between 38,174,447 to 38,387,308 bp on chromosome 1 of the reference genome of *japonica* variety Nipponbare. We found that marker bin150 harbored the "green evolution" gene *semidwarf1* (*sd1*) located at 38,382,382–38,385,504 bp on chromosome 1, encoding gibberellin 20-oxidase, which regulates plant height and grain yield in rice (Spielmeyer et al. 2002). Therefore, we speculate that the *sd1* gene might be a strong candidate for the *qCLN1*, which likely controls coleoptile length for plants germinated under the normal condition.

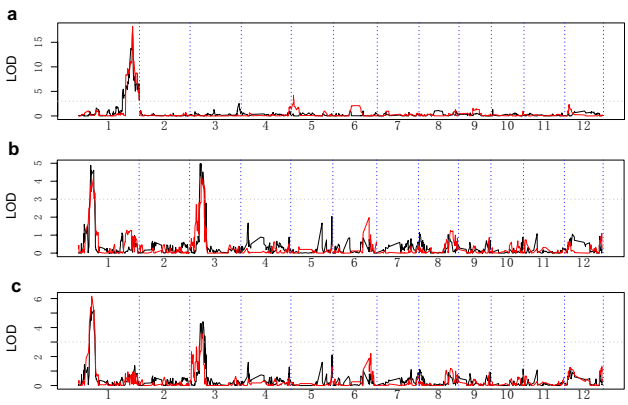

Fig. 2 QTL mapping. QTL mapping results for CLN (**a**), CLA (**b**), and API (**c**). The x-axis represented total 12 chromosome. The y-axis represented LOD value. The black and red lines represented QTL mapping result of replicate 1 and replicate 2, respectively

Under the anaerobic condition, QTLs detected in the two experiments were also co-located (Fig. 2b). Two QTLs, *qAGP1* (*anaerobic germination potential 1*) and *qAGP3* (*anaerobic germination potential 3*), were detected on chromosomes 1 and 3, respectively (Table 1; Fig. 2b). The QTL *qAGP1* was located in bin38 on chromosome 1 and explained 15 % and 14 % of phenotypic variance for replicate 1 and replicate 2, respectively. The QTL *qAGP3* was located in bin346 on chromosome 3 and explained 15 % and 14 % of the phenotypic variance for replicate 1 and replicate 2, respectively. Notably, the chromosome segments of *qAGP1* and *qAGP3* derived from *O. nivara* increased coleoptile length by 0.66 cm (0.63 cm for replicate 2) and 0.64 cm (0.63 cm for replicate 2), respectively, under the anaerobic treatment (Table 1). Additionally, two QTLs related to API were detected at the same chromosome region and exhibited similar genetic effects compared to the QTLs of *qAGP1* and *qAGP3*, respectively (Fig. 2c). These results imply that both coleoptile length and the API value can be used to evaluate AG potential at the germination stage in rice.

Identification of an IL with strong AG potential

To screen for ILs with strong AG potential, we used API values to determine lines with high AG potential. The API value of introgression line Ra25 was 0.308, indicating strong AG potential. The coleoptile length of Ra25 seedlings under the normal and anaerobic condition were 1.14 cm and 1.50 cm, while the coleoptile length of recipient parents (93-11) cultivated under normal and anaerobic

Table 1 QTLs identified under the normal and anaerobic conditions at the germination stage using IL population

	QTL	Chr	Peak marker	Interval region	Physical interval (Mb)	LOD	PVE (%)	Add
Replicate1 (September 2019)	*qCLN1*	1	Bin150	Bin149-Bin152	37.98–38.67	16.9	43	0.16
	qAGP1	1	Bin38	Bin37-Bin45	8.29–11.75	4.9	15	0.66
	qAGP3	3	Bin346	Bin343-Bin351	6.96–10.40	5	15	0.64
Replicate2 (November 2019)	*qCLN1*	1	Bin150	Bin149-Bin152	37.98–38.67	18.2	44	0.17
	qAGP1	1	Bin38	Bin37-Bin45	8.29–11.75	4.2	14	0.63
	qAGP3	3	Bin346	Bin343-Bin351	6.96–10.40	4.2	14	0.63

Chr represents chromosome. PVE indicates percentage of phenotypic variation explained by individual QTL. Add indicates the additive effect of each QTL from *O. nivara*

condition were 1.42 cm and 0.18 cm, respectively (Fig. 3a). The result showed that IL Ra25 had a stronger AG potential than the recipient parent 93-11. Genotype analysis showed that IL Ra25 carried nine chromosomal segments derived from *O. nivara*, comprising six homozygous segments from wild rice and three other heterozygous segments. Notably, the two homozygous segments from wild rice, one on chromosome 1 and the other on chromosome 3, harbored the two QTLs (*qAGP1* and *qAGP3*) for AG potential. (Fig. 3b). Taken together, IL Ra25 is likely an ideal genetic material for fine mapping of target genes and breeding applications in future.

Transcriptome response to anaerobic condition for genes within QTL regions

We determined 494 and 558 genes in the respective *qAGP1* and *qAGP3* regions. A previous study analyzed differential expressed genes (DEG) among six rice genotypes with different levels of anaerobic tolerance (Sheng-Kai et al. 2017). In total, 3597 DEGs were identified by comparing rice samples cultivated under the anaerobic to the normal condition. To further associating these DEG and AG potential, we screened DEGs in our QTL regions. A total of 26 and 36 DEGs were detected in the *qAGP1* and *qAGP3* regions, respectively (Tables S2, S3). Among them, 10 up-regulated and 16 down-regulated genes were detected within *qAGP1* region (Table S2), while 17 up-regulated and 19 down-regulated genes were detected within *qAGP3* region (Table S3). For *qAGP1*, a pyruvate kinase (LOC_Os01g16960), peroxidase precursor (LOC_Os01g16450), and MYB transcription factor (LOC_Os01g16810) might be functionally associated with seedling response to anaerobic stress. For *qAGP3*, a mannose-1-phosphate guanyltransferase (LOC_Os03g16150), cyclase/dehydrase family protein (LOC_Os03g18600), and AP2-like ethylene-responsive transcription factor (LOC_Os03g12950) might be functionally associated with anaerobic stress response.

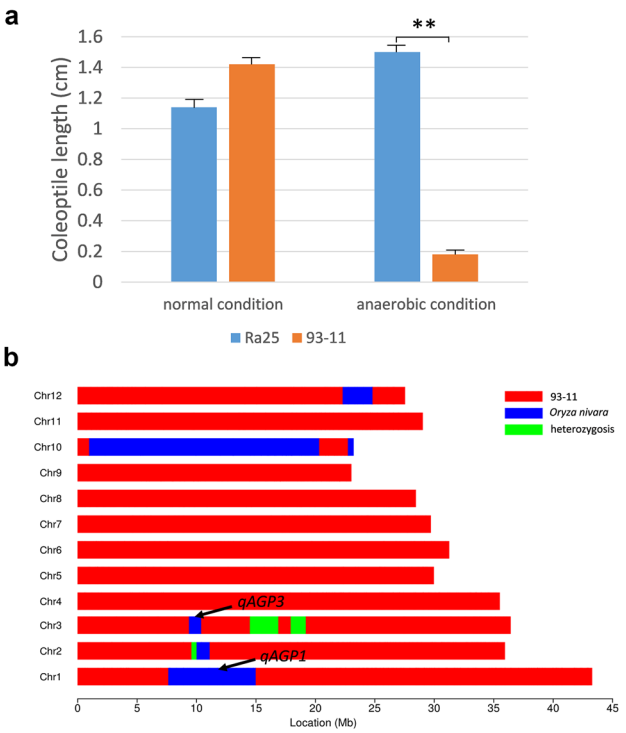

Fig. 3 Phenotype and genotype of Ra25. (**a**) Coleoptile length under norm and anaerobic condition for Ra25 and 93-11 lines. (**b**) Genotype of Ra25. Chromosome segments of 93-11 (Red color), *Oryza nivara* (Blue color), and heterozygosis (Green). The symbol (**) represented significant difference ($p < 0.01$) between coleoptile length of Ra25 and 93-11 under anaerobic condition (color figure online)

Discussion

O. nivara, an unusual wild rice and a close wild progenitor of the Asian cultivated rice, is an important gene pool. There is high genetic diversity among different *O. nivara* accessions (Juneja et al. 2006), which is beneficial to the goal of expanding the genetic diversity of cultivated rice. Some valuable genes responsible for yield production and grain quality have been transferred from *O. nivara* into cultivated rice (Swamy et al. 2012, 2014; Mahmoud et al. 2008). Furthermore, *O. nivara* also shows resistances to grassy stunt virus, bacterial leaf blight, blast fungus, and brown planthopper, as well as drought avoidance (Khush 2000; Brar et al. 1997; Pham et al. 2006; Ali et al. 2010). Therefore, it is very important to investigate the genetic factors in *O. nivara* controlling AG potential.

To date, two main indicators have been used to identify AG potential of rice seeds. One index is the seedling survival ratio after 21 days of submergence under 10 ~ 20 cm water head, which is a standardized method approach developed by International Rice Research Institute (Angaji et al. 2010). Using this method, a number of mapping populations were screened to evaluate AG potential, and several QTLs have been reported (Angaji et al. 2010). However, the survival ratio of this method is labor- and time-intensive. One alternative approach is to measure coleoptile length. The QTLs detected with coleoptile elongation are overlapped with those detected with the survival rate (Hsu et al. 2015). In this study, coleoptile length was measured to map QTL conferring AG potential (Fig. 2). Two main QTLs related to AG potential were detected in this study.

In this study, we measured coleoptile length under anaerobic conditions and used it to represent the ability to germinate anaerobically. The phenotype variance of coleoptile length was normally distributed across diverse accessions, which suggested the quantitative genetic control for this trait.

The coleoptile length of 93-11 was shorter than that of *O. nivara* at the end day (7th day) under anaerobic condition, which indicate that the wild rice, *O. nivara* showed more AG potential than 93-11. Therefore, it is feasible to detect genetic factor controlling AG potential using large linkage population constructing with 93-11 and *O. nivara*.

Three traits, including CLN, CLA, and API, were used to detect QTLs within 131 ILs. QTLs associated with coleoptile length under normal and anaerobic conditions were not co-located (Fig. 2). Interestingly, QTLs detected in anaerobic condition were not co-located with that in normal condition, which indicate that the effect of *qCLN1* was turn off by the anaerobic condition. Furthermore, QTLs associated with CLA and API were located on the same chromosome position (Fig. 2), which might be explained by the high PCC value between the phenotype of CLA and API ($r = 0.82$).

AG tolerance is essential for germination and seedling growth under anaerobic condition. Using the marker-assisted backcrossing strategy, the QTL containing *OsTPP7* gene was transferred into Ciherang-Sub1, which improved the anaerobic tolerance of the near isogenic lines harboring the *SUB1* gene (Mariel et al. 2015). In this study, phenotypic evaluation showed that ILs with *qAGP1* or *qAGP3* from wild rice increased AG tolerance compared to 93-11 (Table S1). Interestingly, ILs with both *qAGP1* and *qAGP3* from wild rice showed more AG tolerance compared to ILs with single alleles (*qAGP1* or *qAGP3*) (Table S1). These results suggest that Pyramiding of favorable QTLs can improve AG potential in rice.

For *qAGP1*, 26 candidate genes were identified. Among them, LOC_Os01g16960, annotated as pyruvate kinase, was up-regulated 4.1-fold in anaerobic condition compared to normal condition. Pyruvate kinase is a key enzyme that regulates and adjusts the final step of the glycolysis pathway (Ambasht et al. 2000; Mattevi et al. 1996). Oxygen deprivation triggers a switch from mitochondrial respiration through the Krebs cycle to fermentative metabolism. Liu et al. (2010) determined a peroxidase precursor (LOC_Os01g16450, LOC_Os01g16152) that was functionally involved in stress response and played major roles in the signaling cascade during germination under submergence. Another candidate gene, a MYB family transcription factor (LOC_Os01g16810) belonging to the family of MYBS1, was up-regulated 8-fold (Lu et al. 2002). A CIPK15-SnRK1A-MYBS1 phosphorylation cascade activated the expression of RAmy3D (Lee et al. 2009; Lu et al. 2002), an important enzyme that is highly expressed when the plant is stressed from oxygen deficiency.

One gene within *qAGP3*, *OsVTC1-3* (LOC_Os03g16150), was up-regulated 3-fold and annotated as mannose-1-phosphate guanyltransferase. Overexpression of *OsVTC1-3* restored ascorbic acid (AsA) synthesis in *Arabidopsis* (Qin et al. 2016). In plants, AsA plays multiple important roles in oxidative stress protection, photoprotection, and development (Qin et al. 2016). Kawano et al. (2002) demonstrated that the content of AsA of an anaerobic insensitive line was declined after submergence for 8 days, and then rapidly recovered after three days of de-submergence. However, sensitive varieties showed slow recovery of AsA content, resulting in slow plant growth. Another candidate gene, *OsPYL* (LOC_Os03g18600) functioned as a positive regulator of the ABA signal transduction pathway (Kim et al. 2012). Overexpression of *OsPYL* led to hypersensitivity to ABA during seed germination, and hence a delay in germination rate (Kim et al. 2012). In our work, *OsPYL* was up-regulated 2.6-fold, which might be responsible for the relative lower growth rate of coleoptiles under the anaerobic condition than that of the normal condition. Another candidate gene, AP2-like ethylene-responsive transcription factor (LOC_Os03g12950) was up-regulated 1.7-fold. The Submergencel (*Subl*) locus includes three ethylene-responsive factor (ERF) transcriptional regulators (Xu et al. 2006). Transcriptome analysis revealed that a set of AP2 family transcriptional regulators were functionally associated with the *SublA-1*-mediated response in plants under submergence (Jung et al. 2010).

Large numbers of QTLs have been detected in a variety of genetic mapping populations. In a BC_2F_2 population developed from a cross between KHAIYAN and IR64, four putative QTLs on chromosomes 1, 2, 11, and 12 explained 51.4% of the phenotypic variance (Angaji 2008). Similarly, another BC_2F_2 population from a cross between Khao Hlan On, an anaerobic germination-tolerant line, and IR64 resulted in the discovery of five QTLs on chromosomes 1, 3, 7, and 9 (Angaji et al. 2010; Septiningsih et al. 2013) reported six significant QTLs on chromosomes 2, 5, 6, and 7 from a $F_{2:3}$ population derived from crossing IR42 and Ma-Zhan Red. Eleven significant SNPs were detected by using an association population (Zhang et al. 2017). In this study, two major QTLs, *qAGP1* and *qAGP3*, were identified. *qAGP1* has been reported in previous studies and was located closely to the QTLs reported in previous studies (Angaji 2008; Angaji et al. 2010). Interestingly, it is the first reporting of *qAGP3* for our study.

Conclusions

131 ILs grown under normal and anaerobic conditions were screened by the phenotype of coleoptile length to investigate AG potential during rice germination (Table S1). Results of QTL mapping showed that two major QTLs were responsible for AG potential (Fig. 2b, c). We found 26 and 36 candidate genes conferring AG potential within *qAGP1* and *qAGP3*, respectively. (Tables S2, S3). In summary, the utilization of *O. nivara*-derived alleles at *qAGP1* and *qAGP3*

enhanced anaerobic tolerance during germination in cultivated rice.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13258-021-01063-6.

Author contributions HZ, XL, YL, and LL designed the research; MH, SL, ZL and LL contributed to the experiments; XL, JM and BF conducted the research; WL, XP and LL analyzed data; LL wrote the paper. LL and XL contributed equally.

Funding This work was supported by the earmarked fund for China Agriculture Research System (CARS-01-14), Finance project of Hunan province-The breeding of rice varieties with low cadmium accumulation (2017XC10).

Compliance with ethical standards

Conflict of interest Licheng Liu, Xiaoxiang Li, Sanxiong Liu, Jun Min, Wenqiang Liu, Xiaowu Pan, Baohua Fang, Min Hu, Zhongqi Liu,Yongchao Li, and Haiqing Zhang declare that they have no confict of interest.

References

Ali ML, Sanchez PL, Yu S, Lorieux M, Eizenga GC (2010) Chromosome segment substitution lines: a powerful tool for the introgression of valuable genes from Oryza wild species into cultivated rice (O. sativa). Rice 3:218–234

Ambasht PK, Kayastha AM (2000) Plant Pyruvate Kinase. Biol Plant 45:1–10

Angaji SA (2008) Mapping QTLs for submergence tolerance during germination in rice. Arf J Biotechnol 7:2551–2558

Angaji SA, Septiningsih EM, Mackill DJ, Ismail AM (2010) QTLs associated with tolerance of flooding during germination in rice (Oryza sativaL.). Euphytica 172:159–168

Baltazar MD, Ignacio JCI, Thomson MJ, Ismail AM, Septiningsih EM (2014) QTL mapping for tolerance of anaerobic germination from IR64 and the aus Landrace Nanhi using SNP genotyping. Euphytica 197:251–260

Bates D, Mächler M, Bolker B, Walker S (2014) Fitting linear mixed-effects models using lme4. Stat Comput 1406:133–199

Brar DS, Khush GS (1997) Alien introgression in riceOryza: from molecule to plant. Springer 35–47

Churchill GA, Doerge RW (1994) Empirical threshold values for quantitative trait mapping. Genetics 138:963–971

Farooq M, Siddique KH, Rehman H, Aziz T, Lee D, Wahid A (2011) Rice direct seeding: experiences, challenges and opportunities. Soil Till Res 111:87–98

Fukao T, Barrera-Figueroa BE, Juntawong P, Peña-Castro JM (2019) Submergence and waterlogging stress in plants: a review highlighting research opportunities and understudied aspects. Front Plant Sci 10:340

Gibbs J, Morrell S, Valdez A, Setter TL, Greenway H (2000) Regulation of alcoholic fermentation in coleoptiles of two rice cultivars differing in tolerance to anoxia. J Exp Bot 51:785–796

Hattori Y, Nagai K, Ashikari M (2011) Rice growth adapting to deepwater. Curr Opin Plant Biol 14:100–105

Hsu SK, Tung C (2015) Genetic mapping of anaerobic germination-associated QTLs controlling coleoptile elongation in rice. Rice 8:1–12

Jiang L, Liu S, Hou M, Tang J, Chen L, Zhai H, Wan J (2006) Analysis of QTLs for seed low temperature germinability and anoxia germinability in rice (Oryza sativa L.). Field Crop Res 98:68–75

Juneja S, Das A, Joshi SV, Sharma S, Vikal Y, Patra BC, Bharaj TS, Sidhu JS, Singh K (2006) Oryza nivara (Sharma et Shastry) the progenitor of O. sativa (L.) subspecies indica harbours rich genetic diversity as measured by SSR markers. Curr Sci India 91:1079–1085

Jung K, Seo Y, Walia H, Cao P, Fukao T, Canlas PE, Amonpant F, Bailey-Serres J, Ronald PC (2010) The submergence tolerance regulator Sub1A mediates stress-responsive expression of AP2/ERF transcription factors. Plant Physiol 152:1674–1692

Kato-Noguchi H, Morokuma M (2007) Ethanolic fermentation and anoxia tolerance in four rice cultivars. Plant Physiol 164:168–173

Khush GS (2000) Rice germplasm enhancement at IRRI. Phillipp J Crop Sci 25:45–51

Kim H, Hwang H, Hong JW, Lee YN, Ahn IP, Yoon IS, Yoo SD, Lee S, Lee SC, Kim BG (2012) A rice orthologue of the ABA receptor, OsPYL/RCAR5, is a positive regulator of the ABA signal transduction pathway in seed germination and early seedling growth. J Exp Bot 63:1013–1024

Kretzschmar T, Pelayo MAF, Trijatmiko KR, Gabunada LFM, Alam R, Jimenez R, Mendioro MS, Slamet-Loedin IH, Sreenivasulu N, Bailey-Serres J (2015) A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nat Plants 1:15124

Lasanthi-Kudahettige R, Magneschi L, Loreti E, Gonzali S, Licausi F, Novi G, Beretta O, Vitulli F, Perata P (2007) Transcript profiling of the anoxic rice coleoptile. Plant Physiol 144:218–231

Lee K, Chen P, Lu C, Chen S, Ho TD, Yu S (2009) Coordinated responses to oxygen and sugar deficiency allow rice seedlings to tolerate flooding. Sci Signal 2:a61

Li H, Durbin R (2009a) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25:1754–1760

Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R (2009) The sequence alignment/map format and SAMtools. Bioinformatics 25:2078–2079

Li H, Ye G, Wang J (2007) A modified algorithm for the improvement of composite interval mapping. Genetics 175:361–374

Loreti E, van Veen H, Perata P (2016) Plant responses to flooding stress. Curr Opin Plant Biol 33:64–71

Loreti E, Valeri MC, Novi G, Perata P (2018) Gene regulation and survival under hypoxia requires starch availability and metabolism. Plant Physiol 176:1286–1298

Lu C, Ho TD, Ho S, Yu S (2002) Three novel MYB proteins with one DNA binding repeat mediate sugar and hormone regulation of α-amylase gene expression. Plant Cell 14:1963–1980

Lu C, Lin C, Lee K, Chen J, Huang L, Ho S, Liu H, Hsing Y, Yu S (2007) The SnRK1A protein kinase plays a key role in sugar signaling during germination and seedling growth of rice. Plant Cell 19:2484–2499

Ma X, Fu Y, Zhao X, Jiang L, Tan L (2016) Genomic structure analysis of a set of Oryza nivara introgression lines and identification of yield-associated QTLs using whole-genome resequencing. Sci Rep 6:1–12

Magneschi L, Perata P (2008) Rice germination and seedling growth in the absence of oxygen. Ann Bot 103:181–196

Mahmoud AA, Sukumar S, Krishnan HB (2008) Interspecific rice hybrid of Oryza sativa× Oryza nivara reveals a significant increase in seed protein content. J Agr Food Chem 56:476–482

Mattevi A, Bolognesi M, Valentini G (1996) The allosteric regulation of pyruvate kinase. Febs Lett 389:15–19

Mariel A, Toledo U, Carlos J, Ignacio I, Septiningsih EM (2015) Development of Improved Ciherang-Sub1 Having Tolerance to Anaerobic Germination Conditions. Plant Bre Bio 32:77–87

Narsai R, Edwards JM, Roberts TH, Whelan J, Joss GH, Atwell BJ (2015) Mechanisms of growth and patterns of gene expression in oxygen-deprived rice coleoptiles. Plant J 82:25–40

Pham TT, Sripichitt P, Chanprame S, Peyachoknagul S (2006) Transfer of drought resistant character from wild rice (Oryza meridionalis and Oryza nivara) to cultivated rice (Oryza sativa L.) by backcrossing and immature embryo culture. Agric Nat Resour 40:582–594

Qin H, Deng Z, Zhang C, Wang Y, Wang J, Liu H, Zhang Z, Huang R, Zhang Z (2016) Rice GDP-mannose pyrophosphorylase OsVTC1-1 and OsVTC1-3 play different roles in ascorbic acid synthesis. Plant Mol Biol 90:317–327

Septiningsih EM, Ignacio JCI, Sendon PMD, Sanchez DL, Ismail AM, Mackill DJ (2013) QTL mapping and confirmation for tolerance of anaerobic conditions during germination derived from the rice landrace Ma-Zhan Red. Theor Appl Genet 126:1357–1366

Sheng-Kai H, Chih-Wei T (2017) RNA-seq analysis of diverse rice genotypes to identify the genes controlling coleoptile growth during submerged germination. Front Plant Sci 8:762

Shingakiwells R, Huang S, Taylor N, Carroll A, Zhou W, Millar H (2011) Differential Molecular Responses of Rice and Wheat Coleoptiles to Anoxia Reveal Novel Metabolic Adaptations in Amino Acid Metabolism for Tissue Tolerance. Plant Physiol 156:1706–1724

Spielmeyer W, Ellis MH, Chandler PM (2002) Semidwarf (sd-1),"green revolution" rice, contains a defective gibberellin 20-oxidase gene. Proc Natl Acad Sci 99:9043–9048

Swamy BM, Kaladhar K, Reddy GA, Viraktamath BC, Sarla N (2014) Mapping and introgression of QTL for yield and related traits in two backcross populations derived from Oryza sativa cv. Swarna and two accessions of O. nivara. J Genet 93:643–654

Swamy BM, Kaladhar K, Shobha Rani N, Prasad G, Viraktamath BC, Reddy GA, Sarla N (2012) QTL analysis for grain quality traits in 2 BC_2F_2 populations derived from crosses between Oryza sativa cv Swarna and 2 accessions of O. nivara. J Hered 103:442–452

Xu K, Xu X, Fukao T, Canlas P, Maghirang-Rodriguez R, Heuer S, Ismail AM, Bailey-Serres J, Ronald PC, Mackill DJ (2006) Sub1A is an ethylene-response-factor-like gene that confers submergence tolerance to rice. Nature 442:705–708

Ye NH, Wang FZ, Shi L, Chen MX, Cao YY, Zhu FY, Wu YZ, Xie LJ, Liu TY, Su ZZ (2018) Natural variation in the promoter of rice calcineurin B-like protein10 (*OsCBL10*) affects flooding tolerance during seed germination among rice subspecies. Plant J 94:612–625

Zhang M, Qing L, Wei W, Xiaojun N, Caihong W, Yue F, Qun X, Shan W, Xiaoping Y, Hanyong Y (2017) Association Mapping Reveals Novel Genetic Loci Contributing to Flooding Tolerance during Germination in Indica Rice. Front Plant Sci 8:678

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.